Diffusion Limitation and Hyperoxic Enhancement of Oxygen Consumption in Zooxanthellate Sea Anemones, Zoanthids, and Corals[1]

J. MALCOLM SHICK

Department of Zoology and Center for Marine Studies,
University of Maine, Orono, Maine 04469-0146

Abstract. Depending on their size and morphology, anthozoan polyps and colonies may be diffusion-limited in their oxygen consumption, even under well-stirred, air-saturated conditions. This is indicated by an enhancement of oxygen consumption under steady-state hyperoxic conditions that simulate the levels of O_2 produced photosynthetically by zooxanthellae in the hosts' tissues. Such hyperoxia in the tissues of zooxanthellate species negates the effect of the diffusive boundary layer, and increases the rate of oxygen consumption; thus, in many cases, the rate of respiration measured under normoxia in the dark may not be representative of the rate during the day when the zooxanthellae are photosynthesizing and when the supply of oxygen for respiration is in the tissues themselves, not from the environment. These results have implications in respirometric methodology and in calculating the rate of gross photosynthesis in energetic studies. The activity of cytochrome *c* oxidase is higher in aposymbiotic than in zooxanthellate specimens of the sea anemone *Aiptasia pulchella*, and this may indicate a compensation for the relative hypoxia in the tissues of the former, enhancing the delivery of oxygen to the mitochondria from the environment.

". . . [zooxanthellae] certainly provide abundant
supplies of oxygen, without which it is just possible that
such immense aggregations of living matter which
constitute a coral reef . . . could not originate and
flourish."

—*C. M. Yonge (1930)*

Introduction

The relatively weak oxyregulatory ability apparent in most anthozoans stems in part from their scant ability to create bulk flow in the seawater surrounding them. In the laboratory, therefore, oxyregulation depends on the amount of convection provided by the experimental apparatus. Under well-stirred conditions, the diffusive boundary layer at the body surface will be thin, and turbulence especially will reduce diffusion gradients. At lower current speeds, the boundary layer thickens, and oxygen uptake becomes more diffusion-limited; this effect is pronounced at low-to-intermediate oxygen partial pressures, so that there is a marked effect of convection on apparent oxyregulatory ability (see Dromgoole, 1978).

The earliest studies of the effect of oxygen partial pressure (P_{O_2}) on the rate of oxygen consumption in anthozoans were made with little or no stirring of the medium, and indicated little or no oxyregulation by *Actinia equina, Anemonia viridis* ($\equiv$ *A. sulcata*) (Henze, 1910), several scleractinian corals (Yonge *et al.*, 1932), *Calliactis parasitica,* and several pennatulids (Brafield and Chapman, 1965). Under well-stirred conditions, the rate of oxygen consumption by most anthozoans increases curvilinearly with P_{O_2}. Recently obtained curves are hyperbolic, and most approach an asymptote at air saturation (*cf.* Mangum and Van Winkle, 1973; Sassaman and Mangum, 1972, 1973, 1974; Shumway, 1978; Ellington, 1982; Tytler and Davies, 1984). In such studies, the rate is often explicitly or implicitly assumed to reach a plateau at air saturation (20.95% O_2, corresponding to 21.23 kPa at one atmosphere), although Mangum and Van Winkle (1973) emphasized that the assumption of a plateau in the commonly used hyperbolic model is not always realized in the data. The assumption has not been

tested in anthozoans, except by Henze's (1910) experiments, where a hyperoxic enhancement of oxygen consumption is evident, albeit under apparently unstirred conditions.

Similarly, investigators of coral productivity assume that respiration proceeds at the same rate in daylight and at night. But when the illuminated zooxanthellae in symbiotic anthozoans are photosynthesizing, oxygen levels in their tissues rise well above air saturation (D'Aoust *et al.*, 1976; Crossland and Barnes, 1977; Dykens and Shick, 1982). Most concerns about the high oxygen levels in the tissues have centered on possible photorespiration or inhibition of photosynthesis in the zooxanthellae (Black *et al.*, 1976; Downton *et al.*, 1976), or on potential oxygen toxicity in the host (D'Aoust *et al.*, 1976; Dykens and Shick, 1982; Shick and Dykens, 1985) and zooxanthellae (Lesser and Shick, 1989).

A role for the zooxanthellae as endogenous providers of oxygen to the host during environmental hypoxia was shown by Shick and Brown (1977), following the demonstration that zooxanthellae affect the spacing between clonal anemones (Fredericks, 1976). Moreover, increased convection in the air-saturated medium increases the rate of oxygen consumption in sea anemones, octocorals, and scleractinian corals (Dennison and Barnes, 1988; Patterson and Sebens, 1989), which implies that a diffusive boundary layer exists and that it impedes the delivery of oxygen to the tissues, even under well-oxygenated conditions. Thus, depending on the extent of external convection—which itself may vary within a coral colony, depending on its hydrodynamic porosity (Chamberlain and Graus, 1975)—oxygen generated photosynthetically *within* the tissues might well negate the effects of the boundary layer and elevate respiration above that measured in darkness at air saturation.

The present paper reports the effects of hyperoxia on oxygen consumption in zooxanthellate anthozoans; the degree of hyperoxia used is within the range known to occur in the tissues of the animals (see Dykens and Shick, 1982). Experimental subjects were chosen to exemplify a range of the size and morphological complexity of polyps, and the growth form of colonies. Finally, the maximum activities of cytochrome *c* oxidase are presented for zooxanthellate and aposymbiotic (lacking zooxanthellae) specimens of *Aiptasia pulchella* maintained under different levels of illumination and oxygenation.

Materials and Methods

Specimens of the sea anemone *Aiptasia pallida* and the zoanthid *Zoanthus sociatus* were collected in the vicinity of the Bermuda Biological Station and were maintained in the station's seawater system prior to use in experiments. Steady-state measurements of oxygen consumption in the dark were made in a BioMetric-CYCLOBIOS twin-flow microrespirometer fitted with Orbisphere model 2120 polarographic oxygen sensors. Millipore-filtered (0.45 μm pore size) seawater (37‰S, 25°C) entering the 50 cm^3 animal chamber was equilibrated sequentially with $O_2:N_2$ mixtures of 21%:79% (normoxia), 50%:50% (hyperoxia), normoxia again, and 10%:90% (hypoxia) using Tylan FC-260 mass-flow controllers. Perfusion of the animal chamber via an LKB MicroPerpex peristaltic pump was varied between 25 and 75 cm^3 h^{-1}, to maintain an oxygen reduction ratio (see Gnaiger, 1983) of about 2–6% between the sensors measuring the oxygenation of seawater entering and leaving the chamber. Water in the chamber was well mixed with a magnetic stirrer situated beneath a perforated plate to which the specimen was attached. Stirring speed was 200 rpm, the maximum that could be used without causing the anemone to collapse or contract. Measurements at each oxygen level were continued for at least 4 h. Values were corrected for blank oxygen consumption at each P_{O_2}.

In a subsequent experiment, a specimen of *Aiptasia pallida* was placed in the 3.5 cm^3 perfusion cell of a ThermoMetric 2277 Thermal Activity Monitor in series with the twin-flow microrespirometer, both regulated at 20°C. The chamber was perfused at a flow rate of 27 cm^{3}h^{-1} for 9 h with 30‰S seawater equilibrated with 21% O_2: 79% N_2, and for an additional 4 h with seawater equilibrated with 50% O_2:50% N_2. Simultaneous fluxes of metabolic heat and oxygen were continuously monitored and analyzed as described in Gnaiger *et al.* (1989).

The clownfish sea anemone *Heteractis crispa*, colonies of the zoanthids *Palythoa tuberculosa* and an unidentified species of *Protopalythoa*, and the scleractinian coral *Stylophora pistillata* were collected from Davies Reef on the Great Barrier Reef, Australia. The sea anemone *Phyllodiscus semoni* was taken from the fouling community in the seawater system at the Australian Institute of Marine Science. Oxygen consumption by *H. crispa*, by the zoanthids and coral, and by large specimens of *P. semoni* was measured in the dark in a closed respirometer (2.3 dm^3) fitted with a Radiometer E5046 oxygen sensor connected to a Radiometer PHM72 Mk2 acid-base analyzer. The specimen was placed on a perforated platform above a large magnetic stirrer operated continuously at 500 rpm, the highest speed that did not disturb *P. semoni*. Polyps of the colonial anthozoans generally remained expanded under this stirring regime. Each specimen was placed in the respirometry vessel, and the seawater (32‰–34‰S, 30°C) bathing it was bubbled with a mixture of 55% O_2:45% N_2 delivered by a Wösthoff type SA/18 gas mixing pump for at least one hour before the chamber was sealed and measurement of oxygen consumption begun. This equilibration period was intended to eliminate transient, diffusional redistribution of oxygen in which *uptake* of oxygen by the relatively hypoxic

body fluids or skeletal pore water of a specimen transferred acutely from the tanks of air-saturated seawater to the respirometer might be interpreted as an initially high rate of *consumption* of oxygen (see Dromgoole, 1978). During the measurements, the specimen was allowed to deplete the oxygen in the respirometer to just below air saturation; the chamber was then flushed, reequilibrated with 55% O_2 for one hour, and the measurements repeated. In cases where the two measurements of oxygen consumption over a particular range of P_{O_2} (either hyperoxia—46.7–45.4 kPa, or normoxia—21.2–20.0 kPa) did not agree to within $\pm 10\%$, the experiment was performed a third time, and the rates of oxygen consumption at both oxygen levels were calculated as the mean of the three measurements at each level. Respiration rates at each oxygen level were corrected for the blank which, in the case of *Protopalythoa* sp., included oxygen uptake by the substrate from which the polyps were removed after the experiment.

The mass of the specimens was variously measured at the end of the respiration experiments, the particular measure being largely a matter of convenience. For *P. tuberculosa* and *Protopalythoa* sp., blotted wet weight ($_wW$) was used, whereas freeze-dried weight ($_dW$) was used for *Z. sociatus* and *P. semoni*. The protein content in individual *A. pallida* was measured by the microbiuret method with bovine serum albumin standards, and in whole colonies of *S. pistillata* by the Bio-Rad Coomassie dye-binding method with bovine gamma globulin standards. As an index of the hydrodynamic porosity of colonies of *S. pistillata*, the ratio of the mean distance between nearest neighbor branches, to the mean branch diameter, was calculated (Chamberlain and Graus, 1975).

Clonal cultures of *Aiptasia pulchella* (obtained from L. Muscatine, University of California, Los Angeles) were maintained in artificial seawater (Instant Ocean, 30‰S) at 25°C. Groups of zooxanthellate anemones were exposed to irradiances of 85 (Dim) or 420 (Bright) μmol $m^{-2}s^{-1}$ under the beam of a Kratos SS1000X 1 kW xenon arc solar simulator (air mass 1 filter). One culture of aposymbiotic anemones (Apo) was maintained in continuous darkness in air-saturated seawater, and another group of aposymbiotic specimens was maintained in the dark in seawater continuously bubbled with 50% O_2 (ApoHiO$_2$). After two weeks of acclimation to these conditions, individual anemones were homogenized (10% w/v) in 100 mM potassium phosphate buffer (pH 7.0). Homogenates were centrifuged at 500 × g for 20 min to remove intact zooxanthellae and animal debris, and cytochrome *c* oxidase (EC 1.9.3.1) in the animal supernatant was assayed at 25°C according to the modified method of Hansen and Sidell (1983), using reduced cytochrome *c* (Sigma Type III).

Results

Specific rates of oxygen consumption (μmol O_2 $g^{-1}h^{-1}$ on the basis of wet or dry weight, or nmol O_2 mg protein^{-1} h^{-1}) by the several species under various oxygen regimes are given in Figures 1–4. Interspecific comparisons of specific rates are not meaningful in the present experiments, owing to the different measurements of mass, which are further complicated by the variable amounts of inorganic material (*e.g.*, sand) in the coenenchyme of the zoanthids.

The rate of oxygen consumption in *Aiptasia pallida* increased at a partial pressure of oxygen approximately twice air saturation (Fig. 1). Although slight, the 11% enhancement of respiration at 50% O_2 is statistically significant (paired $t = 4.04$, df = 4, $P = 0.016$). This is because most of the variance seen in Figure 1 occurred between specimens; all individual anemones showed higher rates of oxygen consumption at 50% than at 21% O_2, which accounts for the significance seen in the paired t-test. The direct calorimetric experiment confirmed that the elevated rate of oxygen uptake reflected an increase in aerobic energy metabolism, as the steady rate of heat dissipation by the anemone increased from 30 μW at 21% O_2 to 33 μW at 50% O_2 (Fig. 1, inset), a 10% rise that closely matched the independent respirometric results. When rates of both heat dissipation and oxygen consumption were steady, the calorimetric-respirometric (CR) ratio was 0.451 μJ pmol^{-1} O_2 at 21% O_2, and 0.463 μJ pmol^{-1} O_2 at 50% O_2. Neither of these values differs significantly from the theoretical oxycaloric equivalent of 0.450 μJ pmol^{-1} O_2 for fully aerobic metabolism (Gnaiger *et al.*, 1989).

The much greater (43%) enhancement, by hyperoxia, of respiration in *Phyllodiscus semoni* (Fig. 2A) is likewise highly significant (paired $t = 6.25$, df = 3, $P = 0.008$). The rate of oxygen consumption in the specimen of *Heteractis crispa* increased by 26% during hyperoxia (Fig. 2B).

Sample sizes for the zoanthids are small (only one or two specimens of each species). Recall, however, that there are two or three measurements (continuous measurements over four hours, in the case of *Zoanthus sociatus*) of oxygen consumption in each species at each oxygen level, so that although this pseudoreplication does not permit statistical analysis, any observed difference in oxygen consumption with P_{O_2} is real. Morphologies of the three species are shown in Figure 3, together with the data. Hyperoxic enhancement of respiration in *Palythoa tuberculosa* averaged 46% (79% in one colony and 23% in a second), whereas the average of repeated measurements on one colony of *Protopalythoa* sp. indicated a slight (8.6%) decline at 50% O_2. Long-term measurements on *Z. sociatus* revealed a 21% increase in a colony of five closely spaced individuals, but essentially no effect (3% increase) of hyperoxia on oxygen consumption in a single polyp subsequently isolated from the colony.

Data are presented separately for two ecomorphs of the scleractinian coral *Stylophora pistillata*, shown in
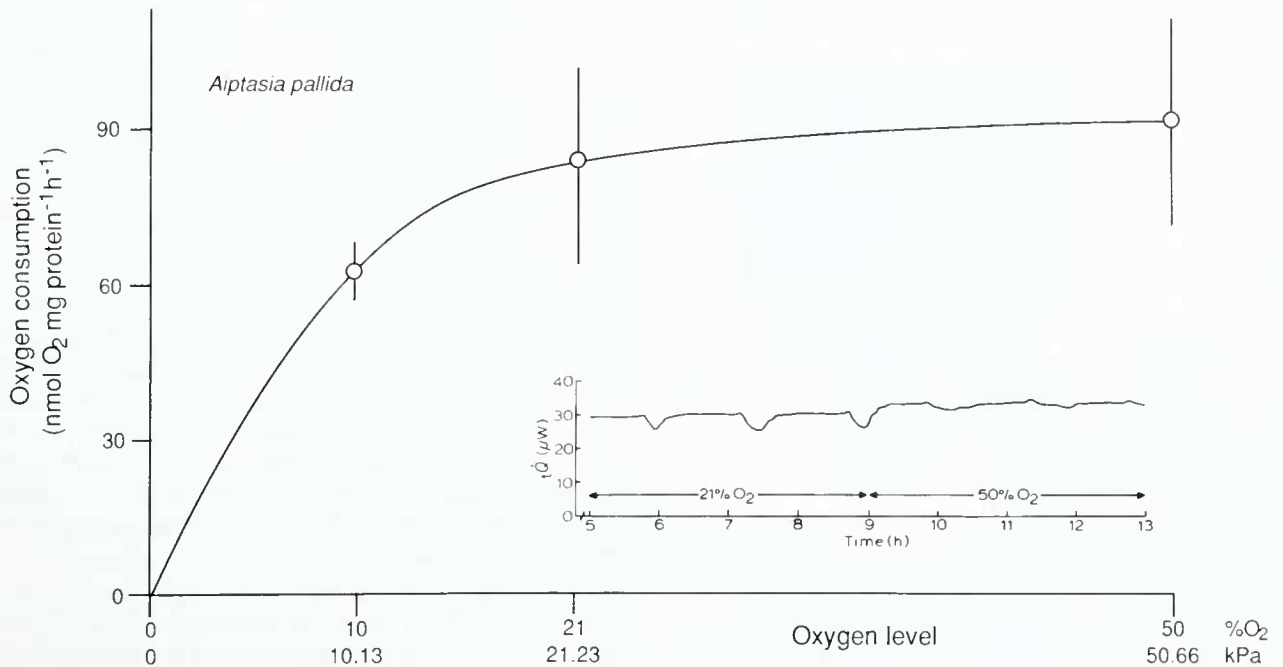
Figure 1. Rates of oxygen consumption in *Aiptasia pallida* (n = 5; mean size = 2.862 mg protein, range = 1.37–4.18 mg protein) under conditions of hypoxia (10% O_2 at inflow to respirometer), normoxia (21% O_2), and hyperoxia (50% O_2). Vertical lines indicate ±1 standard error. Inset: instantaneous heat flux ($_t\dot{Q}$, μW) in a specimen of *A. pallida* (0.788 mg protein) exposed to 21% O_2 and 50% O_2 in an open-flow calorimeter.

Figure 4. Two colonies of the ecomorph having thin, widely spaced branches consistently showed a slight (9.0% and 7.5%) decrease in respiratory rate under hyperoxia, whereas three colonies having thick, closely spaced branches showed a mean 20% hyperoxic enhancement of respiration that was significant (paired t = 5.69, df = 2, P = 0.030). The magnitude of the effect of hyperoxia on oxygen consumption in *S. pistillata* seems to be inversely related to the hydrodynamic porosity of the colony (Fig. 5).

Rates of cytochrome c oxidase activity in the various groups of *Aiptasia pulchella* are shown in Figure 6. Specific activity is expressed in Units per mg protein in the supernatant, each Unit corresponding to 1 μmol cytochrome c oxidized per minute. Analysis of variance indicated a significant effect of treatment on enzymatic activity (F = 7.942, df = 3, 16, P = 0.0018). Individual means were compared using the Student-Newman-Keuls test with a significance level of 0.05.

Discussion

The available studies of effects of hyperoxia on the rate of oxygen consumption in fishes and aquatic invertebrates indicate no enhancement (and perhaps a slight reduction) of the rate relative to that under normoxia. This is largely due to decreases in the ventilatory convection requirement under hyperoxia (Dejours and Beekenkamp, 1977; Toulmond and Tchernigovtzeff, 1984; Berschick *et al.*, 1987), and to the presence of respiratory pigments, both of which stabilize the delivery of oxygen to the tissues over a wide range of external P_{O_2}. Lacking respiratory pigments, and having only weak powers of convection of the external medium, cnidarians are more at the mercy of the Fick equations for diffusive gas exchange. As such, their respiratory exchange must be markedly affected by the flow regime they occupy, and in the case of species harboring algae, by provision of O_2 from those photosynthetic symbionts.

Many anthozoans, especially sea anemones, show behavioral compensations for varying levels of water movement and environmental oxygen supply. A positive relationship exists between the degree of inflation of the hydrostatic skeleton and current velocity in *Metridium senile* (Robbins and Shick, 1980). Although this seems primarily related to prey capture in this suspension feeder, full extension of the column and tentacles also simultaneously maximizes the surface-to-mass ratio and minimizes diffusion distances within the tissues, and thus maximizes oxygen delivery and hence the rate of oxygen consumption (see Shick *et al.*, 1979).

Behaviors that increase the surface area and decrease diffusion distances in the primary gas exchange surfaces are also seen as adaptive short-term responses to hypoxia
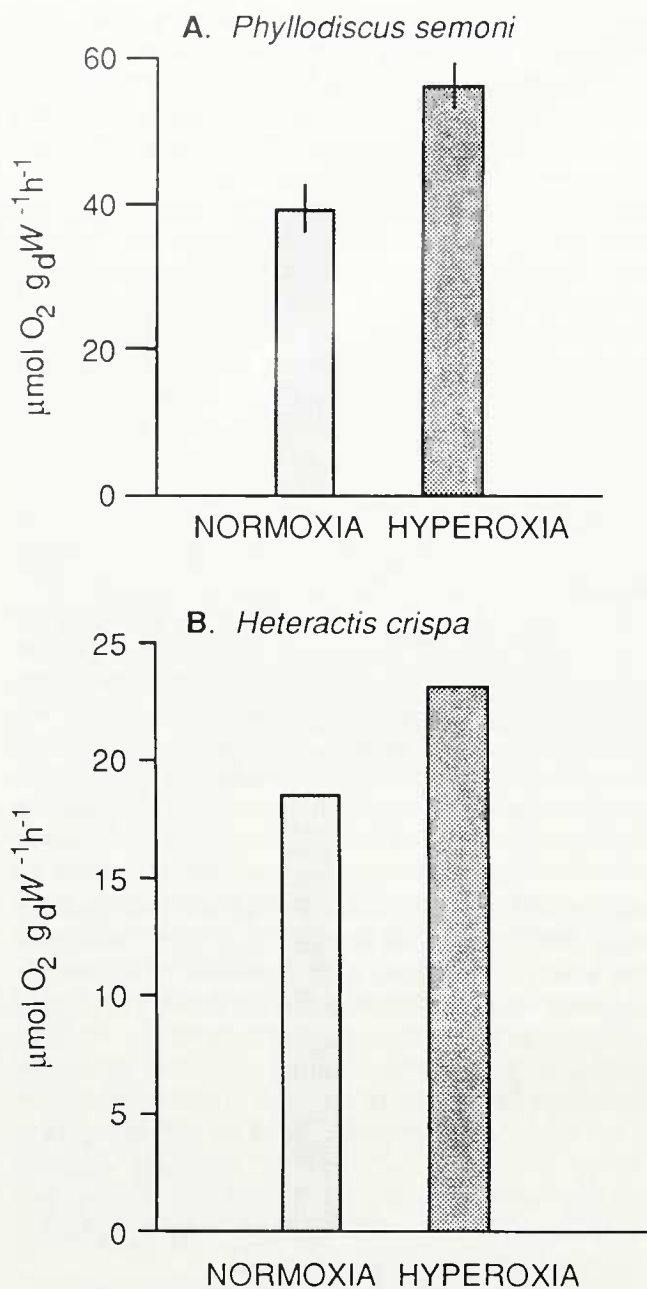
Figure 3. Rates of oxygen consumption in zoanthids. In colonies of (A) *Palythoa tuberculosa* (mean $_wW$ = 47.25 g) and (B) *Protopalythoa* sp. ($_wW$ = 2.89 g), normoxia corresponds to 20.0–21.2 kPa O_2 and hyperoxia to 45.4–46.7 kPa O_2. Vertical lines in (A) indicate ±1 standard error. In *Zoanthus sociatus* (C), the group of 5 individuals weighed 0.011 g $_dW$ and the single polyp 0.003 g $_dW$. Oxygen levels at the inflow to the respirometer were normoxia (21% O_2) and hyperoxia (50% O_2).
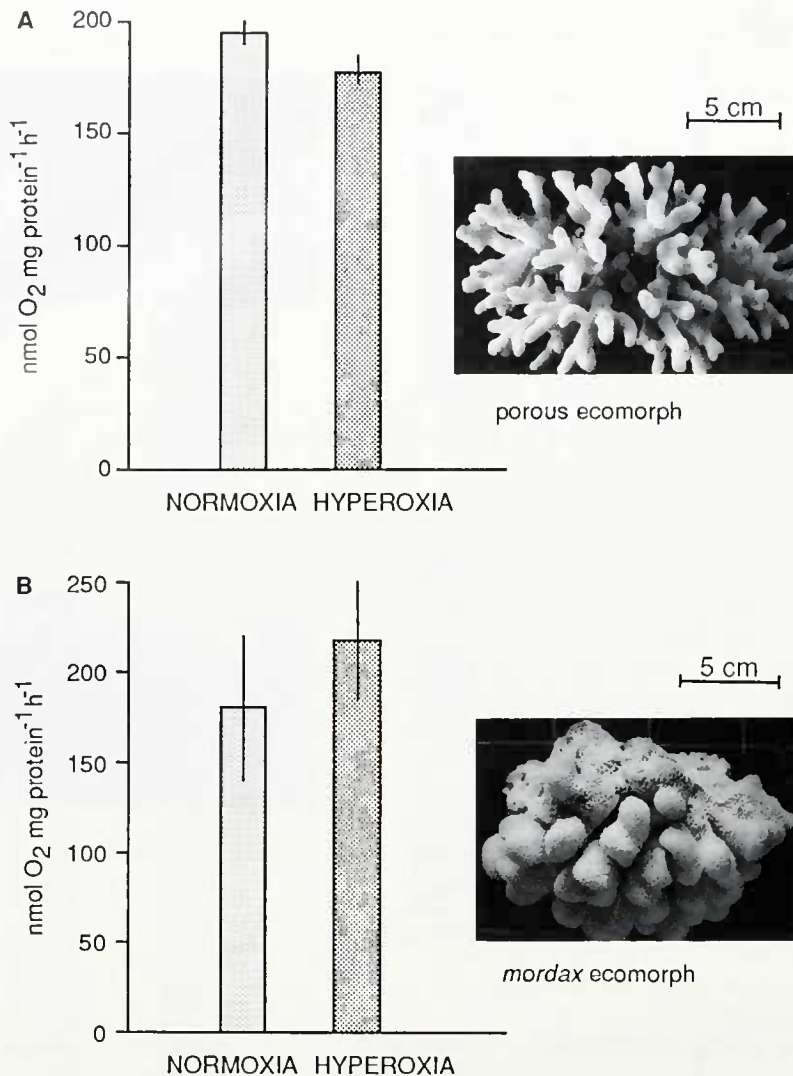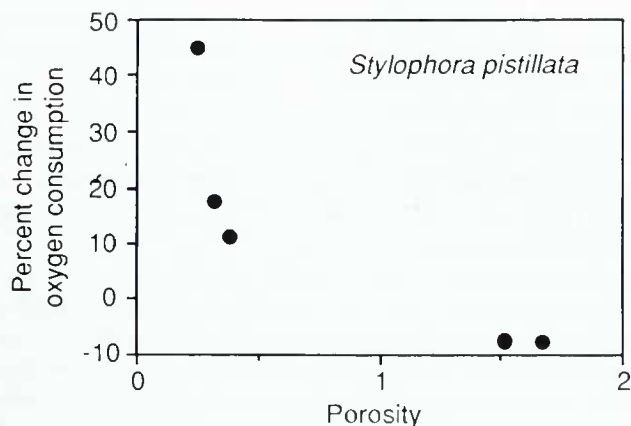
Figure 4. Rates of oxygen consumption in colonies of *Stylophora pistillata* under normoxia (20.0–21.2 kPa O_2) and hyperoxia (45.4–46.7 kPa O_2). (A) In the hydrodynamically porous ecomorph (n = 2), average total colony protein was 369 mg. (B) In the *mordax* ecomorph (n = 3), mean total colony protein was 586 mg. Vertical lines indicate ±1 standard error. Photographs by J.-P. Gattuso.

semoni and *Heteractis crispa,* hyperoxia increases the respiratory rate more than in *A. pallida.* This seems to be related to their much larger size (5 to 10 cm, *vs.* 0.5 cm diameter) and perhaps to their greater morphological complexity. These factors might increase the boundary layer at the body surface, in the first case, because the thickness of the boundary layer around a cylindrical anemone is related to the square root of its diameter (Vogel, 1983). In addition, water flow might be impeded among the numerous pseudotentacles in *P. semoni* and tentacles covering the oral disc of *H. crispa.* Such additional roughness elements may also increase eddy currents and increase the residence time of water in the boundary layer. The existence of such a boundary layer under conditions of forced convection is documented by the experiments of Patterson and Sebens (1989), who found that oxygen consumption by specimens of *Metridium senile* of about the same size as the *Phyllodiscus* in the present study increased two- to threefold as current speed increased from $\approx$7 to 15 cm s^{-1}. Therefore, endogenously produced oxygen (which need not negotiate an external boundary layer) is proportionally more important to large anemones that experience a larger boundary layer that develops even under well-stirred conditions, than to small anemones. Measurements of oxygen flux in large zooxanthellate anemones under normoxic conditions will accordingly underestimate their daytime respiration (and so underestimate gross photosynthesis) more than in small anemones.

Unlike unitary anemones, zoanthids form colonies of

Figure 5. Relationship between the hyperoxia-induced change in oxygen consumption and hydrodynamic porosity in colonies of *Stylophora pistillata*. Porosity was calculated as the ratio of the mean distance between nearest neighbor branches to mean branch diameter. Each point represents one colony.

interconnected polyps that vary in their spacing and aggregate morphology, and thus in their hydrodynamic properties. Such variation has been discussed primarily with respect to the provision of food to these sessile filter feeders (*e.g.,* Koehl, 1977), but the hydrodynamic principles apply to the delivery of O_2 as well. At one morphological extreme, *Palythoa tuberculosa* forms platelike colonies of closely conjoined polyps embedded in a massive, largely inorganic, coenenchyme. Water flow across the colony decreases with increasing distance from the periphery, as kinetic energy is extracted from the flow by skin friction and by form drag of the polyps; thus the interior polyps experience relatively stagnant conditions compared to their clonemates on the edge. Delivery of oxygen to respiring tissues is also impaired by the protective coenenchyme. At the other extreme, *Protopalythoa* sp. from Australia forms loosely aggregated colonies of tall, widely separated polyps connected only at their bases and having less coenenchyme.

Accordingly, *P. tuberculosa* might be expected to be more diffusion-limited in its gas exchange, whereas *Protopalythoa* sp. would perform more like individual small anemones. These predictions are confirmed experimentally, because hyperoxia generates increases in oxygen consumption in *P. tuberculosa* but not in *Protopalythoa* sp. (*cf.* Fig. 3A and B). Moreover, a group of closely spaced polyps of *Zoanthus sociatus* shows hyperoxic enhancement of respiration, whereas a single small polyp responds more like a solitary *Aiptasia pallida* and shows minimal enhancement (Fig. 3C). The difference between individual and colonial respiratory performance in the last case seems to be related to a decrease in free surface area and restriction of water flow between polyps, and hence to a larger effective diameter, with the resultant decrease in water flow toward the center of the colony.

Previous studies concerning physiological effects of water flow around scleractinian coral colonies have been focused primarily on provision of food and removal of waste, although Jokiel (1978) suggested that respiratory exchange is also affected. Dennison and Barnes (1988) and Patterson *et al.* (1990) subsequently demonstrated that respiration under normoxic conditions in *Acropora formosa* and *Montastrea annularis* does increase in moving water. More to the present point, enhancement by light of calcification in *Stylophora pistillata* was suggested by Rinkevich and Loya (1984) to be due to stimulation of (aerobic) metabolism by O_2 produced within the symbiosis by the zooxanthellae. Together these studies indicate that a boundary layer to the delivery of oxygen exists even under normoxic conditions, and that under some circumstances its effects are negated by the production of oxygen within the host's tissues.

Stylophora pistillata exhibits a particularly great diversity of colonial morphologies, to a large extent determined by the flow regime where it occurs (Veron and Pichon, 1976), as well as by photic regime (McCloskey and Muscatine, 1984; Titlyanov, 1987). Chamberlain and Graus (1975) conclude that flow within a branching colony depends entirely on its morphology and on exterior hydrodynamic conditions. Therefore, the prevalence of colonies of *S. pistillata* having thin, widely spaced branches in low energy habitats (Veron and Pichon, 1976; pers. obs.) seems related to the maintenance of adequate flow among the branches in such sheltered areas (and to the avoidance of self-shading in deep water or shaded sites). Although the thick, closely spaced branches of the *mordax* ecomorph of this species would seemingly restrict flow to the interior of the colony, this ecomorph inhabits high energy environments (Veron and Pichon, 1976; pers. obs.). Thus, the ecomorphs of *S. pistillata* exemplify the principle of dynamic similitude—under the appropriate flow conditions, morphologically dissimilar colonies can have similar flow characteristics (Chamberlain and Graus, 1975) and respira-
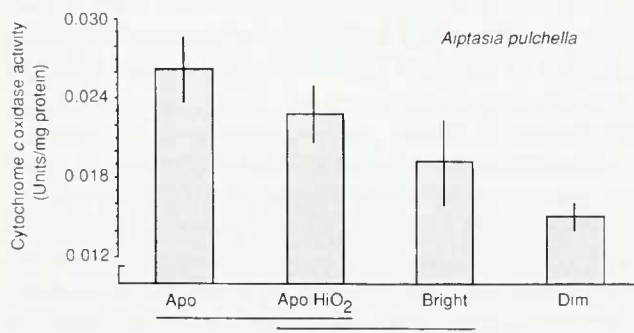
Figure 6. Cytochrome *c* oxidase activity in aposymbiotic and zooxanthellate specimens of *Aiptasia pulchella* (n = 5 in each treatment). Vertical lines indicate ±1 standard error. Horizontal lines underscore groups whose means are not significantly different (*P* > 0.05).

tory rates. The corallites in this species are relatively shallow, so that diffusion distances within the corallum and tissues are short, and probably similar in the different ecomorphs.

In the moderate, turbulent flow in the respirometer, colonies of the hydrodynamically porous ecomorph experience no diffusion limitation, as hyperoxia does not result in an increase in oxygen consumption (Fig. 4A). Similar results are obtained with single branches of colonies (Shick, unpub. data; J.-P. Gattuso, pers. comm.), in which water flow and delivery of oxygen to the polyps is not hindered by any nearby branches. Colonies of the high-energy *mordax* ecomorph, however, do appear to be diffusion limited even under these well-mixed conditions, showing a 20% hyperoxic increase in respiration (Fig. 4B), probably owing to elevated O_2 levels among the interior branches of the colonies. Consequently, the magnitude of the effect of hyperoxia on respiration is inversely related to the hydrodynamic porosity of the colony under these conditions (Fig. 5). Whether this diffusion limitation is more pronounced under unidirectional, laminar flow, or under the turbulent conditions in the present study is unknown, but this is testable with a flow tunnel respirometer.

Compensation for different levels of oxygenation may also be manifested at the cellular level. Lacking zooxanthellae, the tissues of aposymbiotic anemones are hypoxic relative to those of zooxanthellate conspecifics when the latter are photosynthesizing. In *Aiptasia pulchella*, this is associated with a significantly higher activity of cytochrome *c* oxidase (the terminal enzyme in the mitochondrial respiratory chain) in aposymbiotic than in zooxanthellate clonemates. Such an elevation of mitochondrial respiratory capacity in the relatively hypoxic anemones could result from more or larger mitochondria, greater specific activity of cytochrome *c* oxidase per mitochondrion, or a combination of these. Stereological studies of the numbers, distribution, and ultrastructure of mitochondria in anemones under these conditions are in progress. Increasing the numbers of mitochondria (and hence reducing the diffusion distance for oxygen from the cell surface to a respiring mitochondrion) is a correlate of intertidal hypoxic exposure in the anemone *Anthopleura elegantissima* (J. A. Dykens and J. M. Shick, unpubl. data).

The data on aposymbiotic *A. pulchella* cultured under exogenous hyperoxia are consistent with this postulate. Its cytochrome *c* oxidase activity is intermediate to the high activity in relatively hypoxic aposymbiotic clonemates and to the low values in its hyperoxic zooxanthellate clonemates; in the latter case, the major source of oxygen for much of the time is endogenous, from the intracellular zooxanthellae. The lack of effect of irradiance (Dim *vs.* Bright) on cytochrome *c* oxidase activity in zooxanthellate anemones suggests that both groups (maintained at irradiances exceeding the compensation point) experienced similarly high tissue oxygenation during net photosynthesis. However, anemones maintained under bright light tend to have higher cytochrome *c* oxidase activity, which is in keeping with a lower photosynthetic oxygen production owing to their contraction during prolonged high irradiance (Shick, unpubl. data; see also Shick and Dykens, 1984).

An alternative interpretation of the data on cytochrome *c* oxidase is that the activity of this enzyme (and that of the mitochondrial respiratory chain in general) might be lower in zooxanthellate than in aposymbiotic specimens because mitochondria experiencing high oxygen levels have the potential for elevated production of superoxide radicals. Much of the superoxide production in cells occurs via autooxidation of the respiratory chain components NADH dehydrogenase and ubiquinone (Turrens and Boveris, 1980), and its production increases with P_{O_2} (Freeman and Crapo, 1981; Turrens *et al.*, 1982); thus, total mitochondrial production of superoxide radicals in a tissue is a function, both of the concentration of respiratory chain components, and of P_{O_2} (see also Shick and Dykens, 1985). Therefore, the high activity of cytochrome *c* oxidase in aposymbiotic *Aiptasia pulchella*, compared with its symbiotic clonemates, may reflect not simply a compensation for hypoxia in the former, but also an avoidance of oxidative stress in the latter.

The present study demonstrates that, depending on the size and morphology of the species being examined, respiration is variably enhanced by hyperoxia at a level that occurs in the illuminated tissues of zooxanthellate anthozoans, even under well-stirred, turbulent conditions that minimize the thickness of the diffusive boundary layer. Therefore, if the rate of respiration measured in the dark is to be taken as representative of that in the light, then dark respiration must, in some cases, be measured in hyperoxic water. It should be noted that the imposition of exogenous hyperoxia does not affect the thickness of the boundary layer; rather, it steepens the diffusion gradient across the boundary and thus enhances delivery of oxygen to the tissues. Such an enhancement of oxygen delivery simulates the effect of production of oxygen within the tissues, a condition that prevails when the symbiosis is illuminated. Whether and how much this enhances oxygen consumption depends on the size and morphology of the polyps, the hydrodynamic porosity of the colony, and the amount and perhaps the nature (laminar or turbulent) of water flow.

The elevation of respiration by hyperoxia, and the observation that the symbiosis produces more oxygen than it consumes, emphasize that, during daylight, the relevant source of oxygen for respiration is endogenous. Therefore, water movement and the thickness of the diffusive boundary layer around the polyps or colony

may at that time be more relevant to the removal of endogenously produced oxygen, which, at high concentrations, inhibits photosynthesis in the zooxanthellae (Black *et al.*, 1976; Downton *et al.*, 1976) and necessitates greater defenses against oxidative stress in the host and its symbionts (Shick and Dykens, 1985; Lesser and Shick, 1989).

Elevation of respiration by hyperoxia *per se* occurs irrespective of its possible further enhancement by photosynthate translocated from photosynthesizing zooxanthellae (Edmunds and Davies, 1988). Although the provision of oxygen by the zooxanthellae to the host traditionally has been viewed as only supplementary, the current results suggest that the higher oxygen levels in the tissues of zooxanthellate cnidarians reduce the amount of respiratory apparatus (*e.g.*, cytochromes) that the host maintains. This saving may be somewhat offset by the need for higher levels of defenses against oxygen toxicity in zooxanthellate individuals.

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